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Estimation of antioxidant power of human milk from lactating mothers of Rajasthan, India

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Abstract
Human milk supplies all the nutrition and energy that supports the overall development of neonate. It has essential and predominant antioxidant capacity which has a capability to protect infant against all diseases. Present investigation is to analyze the antioxidant power and free radical scavenging activity status in human milk from lactating mothers in the initial period of lactation. Ferric Reducing Antioxidant Power (FRAP) assay and 1, 1-diphenyl- 2-picrylhydrazyl (DPPH) radical method were used for this analysis. In colostrum it was remarkably high level of antioxidant capacity and radical scavenging activity than transitional and mature milks. This study showed the significance of breast milk during the initial days of life and the importance of colostrum milk for neonates.

Keywords: Antioxidant capacity, colostrum, human milk, FRAP, DPPH, radical scavenging activity

Introduction
Mother's milk accommodates all the nutrients which are essential for the distinguished growth and development of infants. It provides all type energy and nutrients that helps to build out the physiological systems, development of brain and immune system of neonate. It possesses, nearly, 72 kcal energy per 100 g of milk, 1.1% protein, 4.2% fat and 7.0% carbohydrate [1].

Reactive oxygen species (ROS) or free radicals are the products of cellular metabolism. They have harmful effects in living organisms as they generate variations in biomolecules such as nucleic acid, carbohydrate, lipids and proteins [2, 3]. Free radical reactions may, in newborns these free radicals cause many harmful effects like kidney failure, retinopathy of prematurity, and intraventricular hemorrhage, necrotizing enterocolitis, bronchopulmonary dysplasia [4]. Antioxidant power is a capability of an organism or a food component to trapped free radicals and reduces their harmful effect.

It has been proven that human milk has essential and extensive antioxidant capacity to save the infant from many infections and diseases [5]. Mother's milk can prevent the dangerous reactions of DNA damage and oxidative stress in infants better than formula milk [6]. Peroxidation caused by free radicals in breast-fed infants is protects from high antioxidant capacity of mother's milk [7]. It also carries ascorbic acid, vitamin E and different enzymes which show the defense mechanism against the oxidation and degradation of biomolecules and reduce harmful effects of oxidative stress [8].

The current study has aimed to analyze the levels of antioxidant capacity and free radical scavenging activity in human milk in the initial lactation period. This investigation was carried out by using simple and reliable experiments, the Ferric Reducing Antioxidant Power (FRAP) assay and 1, 1-diphenyl- 2-picrylhydrazyl (DPPH) radical method.

Materials and Methods
This investigation was approved by the SMS Medical Collage. Written informed consent was obtained from all the enrolled mothers. Total 53 healthy women with normal pregnancy and delivery were involved for this investigation. In our samples set 11 samples of colostrum were collected within the 1-2 days of postpartum, 19 samples were collected within the 7 -10 days postpartum i.e. transitional milk and 23 mature milks samples were collected within the 75-115 days. Fresh milk samples were collected from the lactating mothers in 10 ml sterile containers and were stored at 4 °C immediately after collection.

All the samples were labeled and immediately carried to the Advance Milk Testing Research Laboratory, PGIVER, Jaipur for the antioxidant activity study. Samples were stored at −80 °C until further analysis.

Analysis of antioxidant activity

Ferric Reducing Antioxidant Power (FRAP) Assay

To determine the total antioxidant power of mother's milk samples, a FRAP assay [9] with following modification and precaution (ELISA plate reader) was used. The freshly prepared working FRAP reagent was contain the 300 mmol/L acetate buffer (3.1 g of CH_3COONa and 16 mL of CH_3 OOH- Na-acetate pH 3. 6 sol. should not have any precipitate), 10 mmol/L 2, 4, 6-tripyridyl-s-triazine (TPTZ in 40 mmol/L properly prepared in HCl reagent) and 20 mmol/L $FeCl_3$ in 10:1:1 ratio. (If blue color appears in FRAP reagent after mixing of HCl sol., it is undesirable, so discard it).

Ratio of sample and FRAP reagent should be 10 micro lit. Milk & 300 micro lit FRAP. Incubation temp. 25^0C for 10 minutes. Then measured intensity of blue colored (ferrous tripyridyltriazine complex) at 593 nm using Microplate Reader Spectra Max plus 384 (Molecular Devices, USA). Before reading, centrifuge the reaction mixture to remove precipitate, if any (supernatant to be used for OD reading).

Aqueous solutions of Ascorbic Acid (100–500µM) were used as standards. The data is shown in µmol/L.

DPPH radical scavenging activity

The radical scavenging activity ability (via DPPH test) of mother's milk samples were determined using the method of Brand-Williams [10], with a slight modifications. One ml of 100 µM DPPH methanolic solution was mixed with 250 µL of one Mole Tris-HCl buffer (pH 7.4) and milk samples (25 µL) in the test tube for 30 min at 37 °C room temperature. Mixed the reaction mixture was gently and use the methanol as blank. The absorbance was measured at 517 nm immediately at time t=0 (t0) using Microplate Reader Spectra Max plus 384 (Molecular Devices, USA), then the reaction mixtures were incubated for 30 min in dark at room temperature (37 °C). The absorbance (t30) was taken again at 517 nm. The activity of DPPH was calculated by using formula:

DPPH activity (% scavenging activity) = 100 — (OD at t30 / OD at t0) x100

Results

The antioxidant capacity and radical scavenging activity of lactating mother's milk in distinct periods of lactation was showed in Table 1. The table showed that, colostrum showed significant high level of antioxidant capacity in the FRAP assay in contrast to transitional and mature milks. The antioxidant capacity level decrease from 1102.1 ± 312.6 µmol/L in colostrum to 924.7 ± 112.7 µmol/L in Mature milk. In comparison to the transitional and mature milk, the colostrum have highest radical scavenging activity value (54.2 ± 9.7%) to reduce the stability of free radicals.

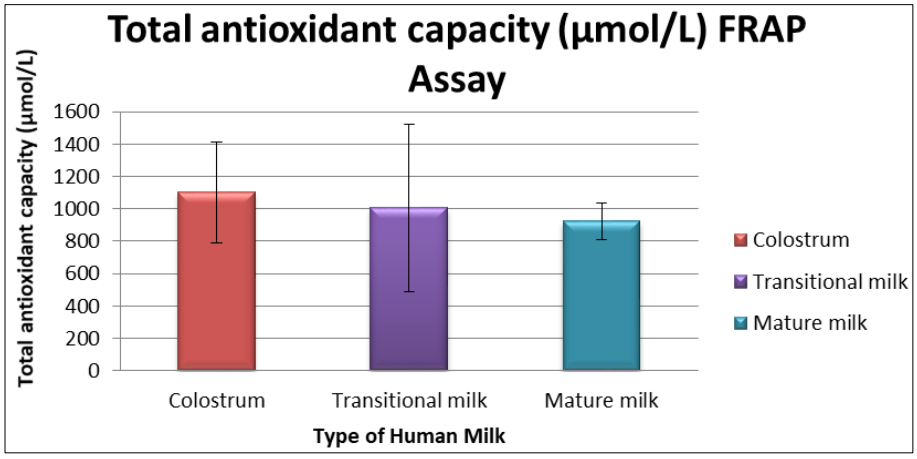

Fig 1: Total antioxidant capacity measured by FRAP assay

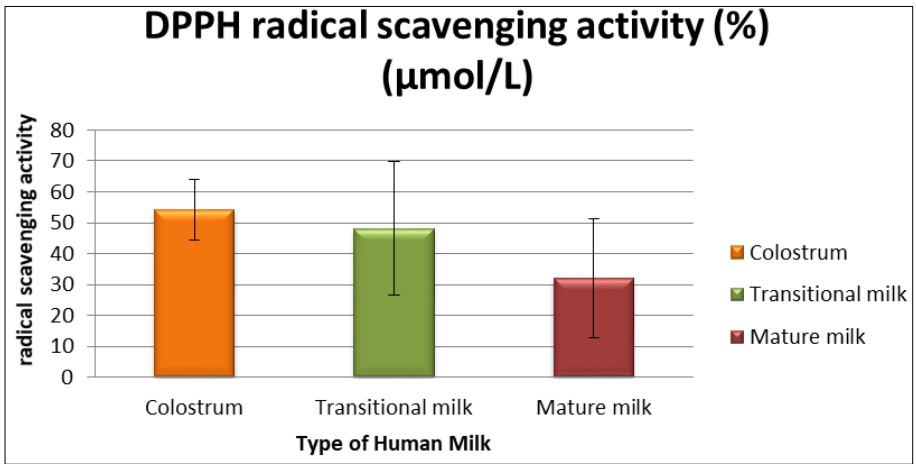

Fig 2: Radical scavenging activity measured by DPPH test

Table 1: The values of Ferric Reducing Antioxidant capacity and DPPH radical scavenging activity in the milk of different lactation period i.e. Colostrum, Transitional milk, Mature milk.

Type of Human Milk	Colostrum	Transitional milk	Mature milk
Period	**1-2 days**	**7 -10 days**	**75-115 days**
Antioxidant capacity (µmol/L)	1102.1 ± 312.6	1005.3 ± 516.3	924.7 ± 112.7
DPPH radical scavenging activity (%) (µmol/L)	54.2 ± 9.7	48.1 ± 21.6	32 ± 19.3

Values are presented as Mean ± SD.

Discussion

In our study, colostrum have highest total antioxidant capacity in contrast to transitional and mature milks and this trend decreasing during the progressive period of lactation. The similar pattern of antioxidant power and scavenging activity was reported [11]. They showed that the colostrum has highest antioxidant power. The antioxidant capability of Expressed breast milk (EBM) decreases along with time and post-refrigeration. The phosphomolybdenum assay was applied by the authors to investigate the antioxidant capacity in the milk samples. A. Zarban *et al*. observed that colostrum have higher antioxidant capacity in contrast to the other type of milk samples. Same results were monitored for radical scavenging activity DPPH test in the human milk samples [5]. S. Yuksel *et al*. reported the high antioxidant capacity in the Colostrum milk samples, and this antioxidant power reduces as the lactation period increases because of changing the need of the growing newbron [12]. C. Matos *et al*. were found that the level of antioxidant power decrease in breast milk samples from 7 days to 4 months [13].

Also, Fidanza *et al*. reported antioxidant capacity in colostrum not significant but its high [14]. The most probable reason for this variation is that because they used oxygen radical absorbent capacity assays to measure the antioxidant capacity in human milk samples. The mother's nutrition plays an important role to decide the level of antioxidant power of breast milk in lactating women. Their food should be containing antioxidant compounds in adequate amount.

In this investigation antioxidant power in the human milk showed a significant decline in the mother's milk samples during progressive period of lactation which can be a standard result. As the lactation period preceded the superoxide activities, catalase activities, the lactoferrin level and the amount nitric oxide decreased, as well as the malondialdehyde level increased. It may be the reason colostrum has a high antioxidant power and as the lactation period increases antioxidants power of breast milk decreases. In this study a major disparity was observed between total antioxidant values. This variation has been related to nutrition given to the lactating mother in whom the food content related to antioxidant compounds was inadequate. Mothers with low values need more attention with regard to their nutrient intake and natural antioxidants compounds during pregnancy as well as lactation period.

Conclusion

The results of this investigation revealed the colostrum have highest antioxidant power and radical scavenging activity. It gives the idea about importance of breast milk after the birth of infant and the significance of colostrum. The information generated in this study is highly suggested that colostrum milk is very important during the initial days of life of a neonate as well as reduction in antioxidant power in the progressive period of lactation in mothers.

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